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16. ABSTRACT

Female CD-1 mice immunized against the bacterium *Streptococcus pneumoniae* type III were exposed to 9-GHz pulsed microwaves (pulse repetition rate 970–1,000, pulse width 1.0 μs, peak power 1 W/cm^2) at an average incident power density of 1 mW/cm^2 (calculated SAR = 0.47 W/kg) for 2 h per day for 5 days. Circulating antibody titers for the microwave-exposed animals were not significantly different from those of the sham-irradiated animals, and there were no differences in any of the hematological parameters analyzed, indicating that 9-GHz pulsed microwaves at 1 mW/cm^2 do not alter the immune response of mice immunized against *S pneumoniae*.

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Brief Communication

Circulating Antibody Response of Mice Exposed to 9-GHz Pulsed Microwave Radiation

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Female CD-1 mice immunized against the bacterium *Streptococcus pneumoniae* type III were exposed to 9-GHz pulsed microwaves (pulse repetition rate 970–1,000, pulse width 1.0 μs, peak power 1 W/cm^2) at an average incident power density of 1 mW/cm^2 (calculated SAR $\simeq$ 0.47 W/kg) for 2 h per day for 5 days. Circulating antibody titers for the microwave-exposed animals were not significantly different from those of the sham-irradiated animals, and there were no differences in any of the hematological parameters analyzed, indicating that 9-GHz pulsed microwaves at 1 mW/cm^2 do not alter the immune response of mice immunized against *S pneumoniae*.

Key words: microwaves, immunology, antibody response, mice

Microwave radiation has been reported to affect the immune response of laboratory animals [Wiktor-Jedrzejczak et al, 1977, 1981; McRee, 1980; Smialowicz et al, 1979]. We have reported stimulation of the circulating antibody response of mice exposed to 9-GHz pulsed microwaves at an incident power density of 10 mW/cm^2 (SAR = 4.7 W/kg) with a concurrent lengthening of the survival time of animals challenged following exposure [Liddle et al, 1980]. The present studies were conducted to determine the response for a lower power density (1.0 mW/cm^2) at 9 GHz.

The animals used and experimental protocol were as described previously [Liddle et al, 1980]. Briefly, female CD-1 mice, 28–35 days of age, were initially sampled for hematology and preimmunization antibody titers by withdrawing 200 λ of blood into heparinized capillary tubes from the ventral tail vein while they were anesthetized with me yfluorane. Four days later they were immunized intraperitoneally (I.P.) with 0.25 μg (0.1 ml) of *Streptococcus pneumoniae* type III purified

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pneumococcal polysaccharide (PPS) (a gift from Eli Lilly and Co., Indianapolis, IN). The animals were then exposed for 2 h per day for 5 days beginning on the day of immunization. On the day following the last irradiation, the animals were again anesthetized and hematological parameters and circulating antibody titers were observed as before. The animals were then challenged (I.P.) with an LD_{50} dose of virulent *S pneumoniae* type III (9×10^3 organisms in 0.1 ml sterile saline). The day of death was observed for 10 days after challenge.

The 9-GHz exposures were conducted in an X-band exposure facility previously described [Liddle et al, 1980]. Eleven groups of four animals each were exposed to 9-GHz pulsed microwaves at an average incident power density of 1 mW/cm^2 (pulse repetition rate 970–1,000, pulse width 1.0 μs, peak power 1 W/cm^2). The SAR was estimated at 0.47 W/kg based on a prolate spheroidal model [Durney et al, 1978]. An equal number of animals were concurrently sham-irradiated.

Antibody titers were measured by the microhemagglutination technique using doubling dilutions in V-bottom disposable plates (Cook Laboratory Products, Alexandria, VA). Sheep red blood cells in Alsever's solution were sensitized according to the method described by Askonas et al [1960] except that 1 ml of type III PPS (300 μg/ml) was used instead of a culture fluid.

Hemagglutination and hematology data were analyzed using Student's t-test.

The results of the hemagglutination procedure for circulating antibody titers are shown in Table 1. The titers (expressed as $\log_2$) for the mice exposed to 9-GHz pulsed microwaves at 1 mW/cm^2 were almost identical to those of the sham-irradiated animals. The results of the hematological analysis are shown in Table 2. Again there were no differences between the exposed and sham-exposed animals.

TABLE 1. Circulating Antibody Titers of Mice Exposed to 9-GHz Pulsed Microwaves at 1 mW/cm^2 (0.47 W/kg)

Group	Number of mice	Titer ($\log_2$) ($\bar{X} \pm$ SE)
Irradiated	42	7.4 ± 0.2
Sham-irradiated	40	7.4 ± 0.2

TABLE 2. Hematology and Differential Results of Mice Exposed to 9-GHz Pulsed Microwaves at 1 mW/cm^2

Test	Microwave-irradiated N = 42 ($\bar{X} \pm$ SEM)	Sham-irradiated N = 40 ($\bar{X} \pm$ SEM)
Red blood cell counts ($\times 10^6$/mm^3)	6.7 ± 0.2	6.5 ± 0.2
White blood cell counts ($\times 10^3$/mm^3)	6.1 ± 0.4	6.3 ± 0.3
Hemoglobin (g/100 ml)	13.9 ± 0.3	13.6 ± 0.3
Hematocrit (%)	36.0 ± 1.4	34.8 ± 1.1
Lymphocytes (%)	82.3 ± 1.3	84.2 ± 1.3
Segmented neutrophils (%)	16.2 ± 1.2	14.5 ± 1.3
Banded neutrophils (%)	0.2 ± 0.1	0.2 ± 0.1
Eosinophils (%)	0.7 ± 0.1	0.8 ± 0.2
Basophils (%)	0.02 ± 0.02	0.0 ± 0.0
Monocytes	0.5 ± 0.1	0.3 ± 0.1

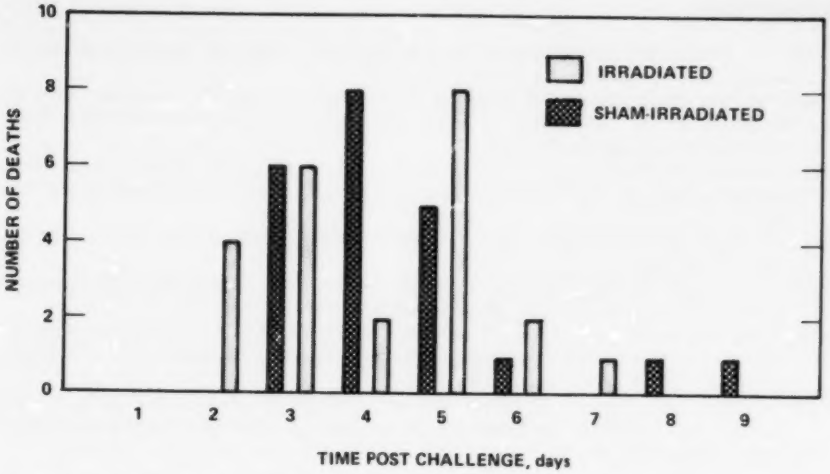

Fig. 1. Mortality by day of death for animals challenged with *S pneumoniae* following exposure to 9-GHz pulsed microwaves at 1 mW/cm^2.

The percentage mortality for the animals challenged with virulent *S pneumoniae* following radiation was 54.8% (23/42) compared to 56.4% (22/39) for the sham-irradiated animals ($\chi^2 = 0.022$, $p \simeq .99$), indicating that there was no evidence of increased protection in the microwave-exposed animals. In addition to measuring the percentage mortality, the day of death was also analyzed to determine if there might be a shift in the mortality pattern. These results are shown in Figure 1. There was a tendency for the sham-exposed animals to survive longer than the microwave-exposed animals (4.45 ± 1.57 vs 4.04 ± 1.46) ($\bar{x}$ ± SD), but this difference was not significant ($0.9 > P > 0.5$, Mann-Whitney U-test).

Rectal temperatures were taken immediately after exposure on both the microwave- and sham-irradiated animals. The measured values were 38.2 ± 0.8 °C for the microwave-exposed animals and 38.2 ± 0.5 °C for the sham-exposed animals, indicating that the animals were not thermally stressed.

The results of this study indicate that 9-GHz pulsed microwaves at an incident power density of 1 mW/cm^2 (~0.47 W/kg), 2 h/day for 5 days do not alter the circulating antibody response of mice previously immunized with type III *S pneumoniae* PPS. In a previous study [Liddle et al, 1980], we reported a significant increase in the circulating antibody titers and a delay in time of death of mice exposed to 9-GHz pulsed microwaves at an incident power density of 10 mW/cm^2 (~4.7 W/kg) using the same exposure and immunization protocol as in the present study. The PPS solution used in this study was the same as that used for the last part of the previous study, and the antibody titers for the sham-exposed animals for these two groups were compared and found to be statistically similar. These studies suggest that the threshold in mice for an increased circulating antibody response to type III *S pneumoniae* PPS from exposure to 9-GHz pulsed microwaves lies between 1 and 10 mW/cm^2 (0. 47 and 4.7 W/kg).

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